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20010019 5 January 2001 (05.01.2001) **FI**(71) Applicant (for all designated States except US): **BIOHIT OYJ** [FI/FI]; Laippatie 1, FIN-00880 Helsinki (FI).

(72) Inventors; and

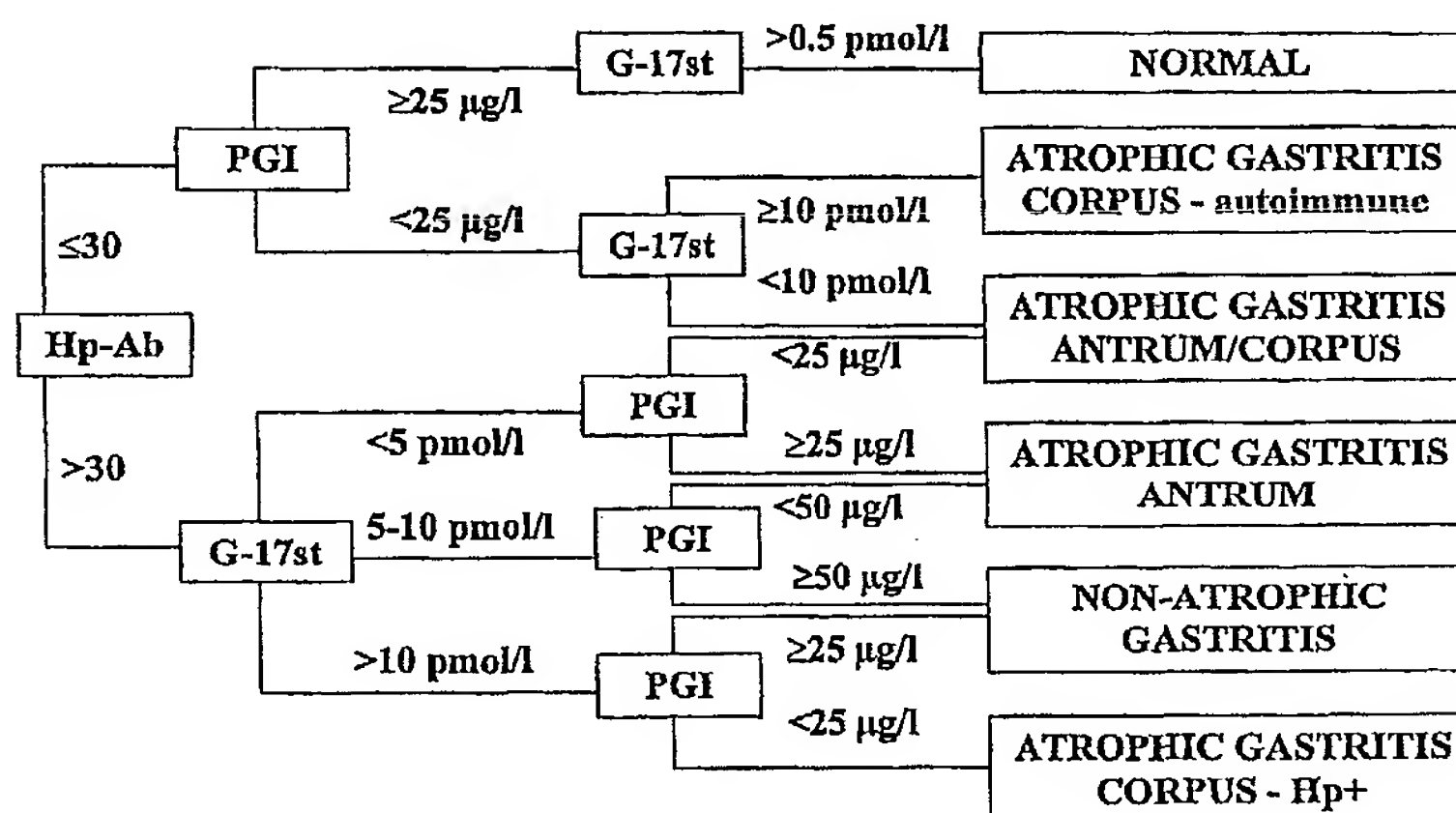
(75) Inventors/Applicants (for US only): **SUOVANIEMI, Osmo** [FI/FI]; Kulopolku 6, FIN-00570 Helsinki (FI). **HÄRKÖNEN, Matti** [FI/FI]; Harjuviita 14 C 24, FIN-02100 Espoo (FI). **TIUSANEN, Tapani** [FI/FI];

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(54) Title: A METHOD FOR DIAGNOSING ATROPHIC GASTRITIS



(57) Abstract: The invention concerns a method for assessing the condition of the gastric mucosa, especially for diagnosing mucosal gastric changes, such as atrophic gastritis, in a subject, by assaying the analytes pepsinogen I (PGI), gastrin and a marker for *Helicobacter pylori* infection, the method comprising - measuring from a sample of said subject the pepsinogen I and gastrin concentration, and, in addition, determining the concentration or presence of a marker for *Helicobacter pylori* (Hp-marker), and - entering the data so obtained for said analytes in a data processing means comprising an operating system, means for transceiving and processing data, said data processing means being adapted to perform the steps of - comparing a measured concentration value for an analyte to a predetermined cut-off value for said analyte, to obtain a combination of comparison results which is specific for the subject tested, and generating information in response to the said combination of comparison results, and optionally other data entered. The invention is also directed to a kit and to computer program product especially for use in the method according to the invention.



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## A METHOD FOR DIAGNOSING ATROPHIC GASTRITIS

### FIELD OF THE INVENTION

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The present invention is directed to a method for assessing the state or condition of the gastric mucosa, especially for diagnosing high-risk changes in the gastric mucosa, especially atrophic gastritis, in a subject. In the method the concentration of specific analytes, namely the pepsinogen I concentration, the gastrin (preferably

10 gastrin-17) concentration as well as the concentration or presence of a *Helicobacter pylori* marker, and optionally also the pepsinogen II concentration, is determined in the subject, and data processing means are used to process the data obtained and to provide information, for example, in the form of a diagnosis, or suggestions for further treatment or investigations.

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### BACKGROUND OF THE INVENTION

Although the occurrence of new cases of gastric cancer has diminished in the recent years, gastric cancer is still one of the most common malignancies. In

20 Finland, appr. 250 to 300 new cases of cancer/one million people/year are registered. In the age group of people above 50, there are an estimated 2350 cases of stomach cancer, which is about 3 per mille of the age group population (Finnish Cancer Registry - The Institute for Statistical and Epidemiological Cancer Research 1993). In addition to Finland, there is a high gastric cancer incidence in Iceland,

25 South America and especially in Japan and China.

The prognosis of gastric cancer is usually poor, as there is no specific treatment. Presently the only possibility of successfully treating gastric cancer is its early detection and total removal surgically.

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Gastric cancer does not necessarily give any symptoms in its early stages. The late appearance of symptoms naturally delays the patient from seeking treatment. On the other hand, the clinical findings in the early stage of gastric cancer are often

non-specific. The primary diagnostic method for gastric cancer is presently gastroscopy and biopsies, cell and aspiration cytology associated therewith. As routine gastroscopies are carried out in order to examine symptoms, such as pain in the upper abdomen or bleeding of the gastrointestinal tract, a symptomatic gastric cancer discovered in this manner is often already far advanced and thus inoperable. Attempts have also been made at improving primary diagnostics with various immunological methods, but no sufficiently specific immunological method has been successfully developed.

It is a primary object to find the means by which it would be possible to identify within the general population easily and with moderate costs those persons which might be suffering from gastric cancer in its initial stages. After identification these persons should immediately be examined by gastroscopy. At the same time those persons could be identified which exhibit premalignant gastric changes which need to be followed up.

Gastric cancer can be preceded by a number of different gastric diseases or conditions (so called precancerous conditions), which are chronic atrophic gastritis, pernicious anaemia, ventricular ulcer, gastric polyposis and the Ménétrier disease (giant hypertrophic gastritis). Clearly identifiable changes of the mucosa are dysplasia and adenoma. The said conditions are associated with an appr. 4 to 5 fold relative cancer risk, as compared to the general population. It has been established that in almost all diseases the risk is mediated over chronic atrophic gastritis.

Chronic gastritis means a prolonged inflammatory condition of the gastric mucosa. The disease can coarsely be divided into the so-called superficial and the atrophic form. In superficial gastritis, the inflammatory cell infiltration is concentrated below the surface epithelium. In case the inflammation progresses and diffuses between the specific gastric secretory glands, one refers to chronic atrophic gastritis. In such a case, the normal glandular structures of the gastric mucosa are at least partly substituted by metaplastic changes.

The relative risk of gastric cancer in patients suffering from atrophic gastritis in the corpus area of the stomach, has been estimated, as calculated from the Finnish cancer statistics, to be about 4- to 5-fold as compared to persons having a healthy mucosa. In addition, there is a risk for falling ill with pernicious anaemia due to intrinsic factor deficiency and B12 vitamin absorption disturbance. In severe atrophy of the antrum area, the risk is even 18-fold. If atrophic changes appear both in the antrum and the corpus area (pangastritis), the risk can increase to even 90-fold (Sipponen, P, Kekki, M, Haapakoski, J. Ihamäki, T & Siurala, M (1985) Gastric cancer risk in chronic atrophic gastritis: statistical calculations of cross-sectional data. Int J Cancer 35:173-77).

*Helicobacter pylori* is a spiral shaped, gram-negative bacterium which thrives in the mucus in the immediate vicinity of the surface epithelial cells of the gastric mucosa and in the cell interstices. The bacterium apparently is transmitted perorally from one person to the other. The effect of the bacterium on the gastric mucosa is an inflammation reaction, which is mediated over a complement by liberating strong inflammation mediator substances. After the acute stage, the inflammation is transformed into chronic gastritis. In patients suffering from chronic gastritis, in 70 to 90 % a *Helicobacter pylori* infection can be established (Calam, J (1994) *Helicobacter pylori* (Review) Eur. J. Clin Invest 24: 501-510). As *Helicobacter pylori* infection and chronic gastritis in the stomach are closely associated, it has been stipulated that this bacterial infection could be one etiological factor in the development of stomach cancer. It is for this reason possible that eradication of the *Helicobacter pylori* bacteria in the initial stages of the infection, could prevent the development of atrophy associated with chronic gastritis, and thus reduce the cancer risk and the risk of peptic ulcers.

The publication WO 96/15456, which is included herein for reference, discloses a method for screening for the risk of cancer by determining the concentration of the analytes pepsinogen I and gastrin-17 from a serum sample of a subject. According to the said publication, the so determined concentration values are then compared

to a cut-off value and a reference value for each analyte. A serum pepsinogen I concentration below the cut-off value for pepsinogen I in combination with a gastrin-17 concentration value above the upper reference limit indicates severe atrophy of the corpus area of the stomach. A serum gastrin-17 level below the cut-off value for gastrin-17 in combination with a pepsinogen I value above the cut-off value for pepsinogen I on the other hand indicates atrophy of the antrum area of the stomach. In case the serum pepsinogen I is below the cut-off value for pepsinogen I and the gastrin-17 level is at the lower limit of its reference value, this is an indication of severe atrophy in the whole stomach, *i.e.* of atrophic pangastritis.

According to an embodiment disclosed, the said tests may be combined with a test for *Helicobacter pylori* antibodies.

According to the said WO-publication, the method can be supplemented with a so-called protein stimulation test, according to which a blood sample is taken in the morning after fasting, whereafter the patient eats a protein-rich standard meal and blood samples are taken at 15 minute intervals for two hours. The maximal increase is evident after appr. 20 minutes. In case the atrophy is located in the antrum, there will be a strongly reduced response in this test. When the atrophy is located in the corpus, the response will be normal or increased, whereas atrophy of the whole mucosa leads to a reduced response.

The WO-publication WO 00/67035, which is included herein for reference, discloses a method for assessing the risk of peptic ulcer by determining quantitatively the concentration of serum pepsinogen I and serum gastrin-17. According to this method, if both the measured serum pepsinogen I and gastrin-17 values are high, above the upper limit of their respective reference values, or the serum pepsinogen I value is above the upper limit of its reference value in combination with a gastrin-17 value within the reference range or below its cut-off value, this is an indication of an increased risk of peptic ulcer.

Methods are known in the art for measuring the concentrations of the various analytes, and there are also commercially available kits for this purpose. Some exemplary methods for carrying out the said determinations are described in the WO-publication 96/15456 as well.

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## SUMMARY OF THE INVENTION

The object of the invention is a method for assessing the state of the gastric mucosa, especially for diagnosing mucosal gastric changes, such as atrophic gastritis, in a subject, by assaying the analytes pepsinogen I (PGI), gastrin and a marker for *Helicobacter pylori* infection, the method comprising

10 - measuring from a sample of said subject the pepsinogen I and gastrin concentration, and, in addition, determining the concentration or presence of a marker for *Helicobacter pylori* (Hp-marker), and

15 - entering the data so obtained for said analytes in a data processing means comprising an operating system, means for transceiving and processing data, said data processing means being adapted to perform the steps of

- comparing a measured concentration value for an analyte to a predetermined cut-off value for said analyte, to obtain a combination of comparison results which is

20 specific for the subject tested, and generating information in response to the said combination of comparison results, and optionally other data entered.

The data processing means can comprise a display on which the generated information is displayed. The generated information advantageously relates to a

25 diagnosis or a suggestion for treatments or further investigations and/or tests based on the comparison result obtained.

The invention is also directed to a kit and to a computer program product especially for use in the method according to the invention.

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The invention is also directed to a kit comprising means for measuring, from a sample, the pepsinogen I and/or gastrin concentration, and/or means for

determining the concentration or presence of a *Helicobacter pylori* marker, as well as a computer program product embodied on a computer readable medium and comprising computer code means adapted to perform the steps of comparing a measured concentration value of an analyte to a predetermined cut-off value for said analyte, combining the results of comparison to a combination of comparison results, and providing information in response to said combination and optionally to other entered data, when run on a computer.

Yet another object of the invention is a computer program product embodied on a computer readable medium and comprising computer code means adapted to perform the steps of comparing measured concentrations of an analyte to a respective cut-off value for said analyte, combining the results of comparison to a combination of comparison results, and providing information in response to said combination and optionally to other entered data, when run on a computer.

#### DETAILED DESCRIPTION OF THE INVENTION

The process of comparing the measured value to a cut-off value of an analyte is aimed at establishing if the measured value of the analyte is greater than, equal to or smaller than the respective cut-off value, and thus to generate specific combinations of comparison results for the analytes, based on which combination specific information will be generated and for example displayed on a display.

Thus according to the invention, for each subject to be tested, the data processing means generate a specific set of comparison results, which set is obtained by comparing a measured value to a predetermined cut-off value for the analytes, in any given order, or simultaneously. In the context of the invention, a subject means a mammal, such as a human, or an animal, such as a pet, e.g. a dog.

The present invention thus includes in a first step a method for determining at least a *Helicobacter pylori* marker, pepsinogen I (PGI) and gastrin.

The marker or indicator for *Helicobacter pylori* infection can thus, according to the invention, be for example a *Helicobacter pylori* antibody, the value of which can be measured from a body fluid sample. Such a sample is advantageously a serum sample, but can also be a saliva, urine or lacrimal fluid sample, e.g. commercial kits being available for measuring the antibody value. The cut-off value for *Helicobacter pylori* antibodies can easily be determined by a person skilled in the art. In one embodiment of this invention we have used a value of 30 EIU as a cut-off for indicating the presence or absence of a *Helicobacter pylori* infection. Specific antibodies to *Helicobacter pylori* can also be measured using western blot.

Another alternative is to evaluate the presence of a *Helicobacter pylori* infection by determining the presence of the antigen itself. Such measurement can for example be carried out on a stool sample from a patient, and assays, such as enzyme immunoassays are commercially available for this purpose (cf. for example Lancet 1999; 354, 30-33). It is also possible to determine the presence of the antigen from the breath of a patient, using the well known techniques of measuring the carbon dioxide content, the formation of which from labeled urea is catalyzed by *Helicobacter pylori* bacteria, and systems for this assay are also commercially available (e.g. Heliprober<sup>™</sup> by Noster AB, Sweden). Another alternative is to determine the presence of the antigen in a biopsy sample taken during endoscopy from the stomach of a patient. In this alternative the tests are either positive or negative for antigen, and a presence of antigen is taken as being indicative of a *Helicobacter pylori* infection. The result of the test is suitably entered in the data processing system as a boolean parameter.

Pepsinogen and gastrin are preferably determined from a body fluid sample, especially from a serum sample, or from a urine, salivary, or lacrimal fluid sample.

The method includes measuring the pepsinogen I analyte, but according to an embodiment of the invention it is also possible the measure, in addition, the concentration of the pepsinogen II analyte (PGII), and using the ratio PGI to PGII

instead of PGI, or in addition thereto as the value to be compared to a predetermined cut-off value. Pepsinogen I and also the ratio of pepsinogen I to II tend to decrease linearly with worsening of the atrophy of the corpus mucosa.

5 In the method according to the invention a first lower cut-off value for pepsinogen I is typically used, which is the 'normal' cut-off value for differentiating between normal and atrophic corpus mucosa. In addition a second higher cut-off value for pepsinogen I can be used, such higher value, when the gastrin concentration is in its cut-off range indicating only slightly stimulated gastrin activity, defining the  
10 cut-off between a non-atrophic (incl. corpus) gastritis (pepsinogen I above second cut-off), and only mildly atrophic corpus mucosa (pepsinogen I below second cut-off) in combination with antrum atrophy. The said second cut-off value can easily be determined by the person skilled in the art.

15 The concentration of gastrin, and especially of gastrin-17, likewise decreases with worsening of the atrophy of the antrum mucosa. For gastrin, a cut-off range can be used. This range has a lower cut-off limit, values below which indicate atrophy of at least the antrum, as well as an upper cut-off limit, values above which can indicate normal, non-atrophic gastritis or an atrophic corpus mucosa. Values in  
20 the range between the lower and the upper value can indicate atrophy at least in the antrum area, or, in combination with a high pepsinogen value (above the second higher cut-off value), also non-atrophic gastritis, as discussed above. In addition, a third cut-off value, a lowest cut-off value for gastrin can be applied for indicating a normal mucosa when pepsinogen I is equal to or above its first cut-off value.

25 The gastrin analyte can be total gastrin, that is essentially the sum of gastrin-34 and gastrin-17, but according to a preferred embodiment of the invention, gastrin-17 is measured.

30 As mentioned earlier, the methods for measuring pepsinogen I, pepsinogen II and and gastrin are well known in the art. The measurements are usually immunological methods, using mono- or polyclonal antibodies to the said analytes.

The detection methods for use include, for example, measuring absorbance, fluorescence or luminescence. It is also possible to carry out all three analyte measurements simultaneously, for example on the same microplate, in different wells or in the same well thereon, which provides for an especially convenient method.

The analyte values so obtained are then entered in a data processing means, such as a data terminal, which has the necessary operating system and which can be provided with means for data input and data output and which comprises means for comparing a measured analyte concentration to a predetermined cut-off value for the analyte. The data can be entered using any data terminal equipment with a proper user interface (UI) or by reading data from a data carrying and storing medium, or by receiving data from a data providing system, via data transmission means and data communication networks, such as internet.

The user interface is thus the means by which the user can communicate with the information providing system, and by means of which data is entered, validated and whereto generated information is reported or displayed, or for further transmission, for storage, or to be printed on a printer.

The concept of cut-off values in assays involving the determination of analyte concentrations is well known to the person skilled in the art, and it generally means a value or a set of values chosen as a limit between the reference values (normal values) and the abnormal values for the test in question. Such cut-off values are method-specific and depend on the specificity and sensitivity chosen for the test method used for the determination of the analyte concentrations, see for example William J Marshall, Clinical Chemistry, Third Edition, 1995, Mosby.

The cut-off values can be entered into the data processing system prior to entering the measured analyte values, or they can be pre-stored in the data processing system. According to the invention it is also contemplated to modify the cut-off.

values when carrying out the method, in case it is desired change the sensitivity or specificity of the method.

According to the invention the data processing means perform a comparison  
5 operation, according to which the various analyte concentrations measured are compared to a respective cut-off value or cut-off range for said analyte, the comparison results obtained are combined, and information in response to the said combination of comparison results and optionally to other or additional data or parameters entered in the data processing means, is generated, which information  
10 can be displayed for example on a display on the user interface.

Information created by the data processing means can take the form of a diagnosis displayed in a window on a display, or it can take the form of suggestions for treatment, or suggestions for further investigations or tests, displayed in the same  
15 or in a different display window.

Thus in accordance with the invention, depending on the specific combination of comparison results obtained, a main diagnosis can refer to 'normal (mucosa)', 'atrophic corpus gastritis' (optionally of autoimmune type), 'atrophic antrum and  
20 corpus gastritis' (pangastritis), 'atrophic antrum gastritis', or 'non-atrophic gastritis, supplemented with information relating to the presence or absence of a *Helicobacter pylori* infection.

The following Table includes specific combinations of comparison results  
25 associated with the different diagnoses, when the analytes are Hp-marker, such as a Hp-antibody, pepsinogen I and gastrin.

In the Table,

Hp-co means the cut-off value for the Hp marker

30 P-coI means the first cut-off for pepsinogen I

P-coII means the second cut-off value for pepsinogen I

G-coI means the lower cut-off value for gastrin

G-coII means the upper cut-off value for gastrin.

	Diagnosis	Hp-marker	PGI	gastrin
5	Normal	$\leq$ Hp-co	$\geq$ P-coI	any
	Atrophic corpus gastritis	$\leq$ Hp-co	$<$ P-coI	$\geq$ G-coII
	Atrophic antrum/corpus gastritis	$\leq$ Hp-co	$<$ P-coI	$<$ G-coII
	Atrophic antrum/corpus gastritis	$>$ Hp-co	$<$ P-coI	$<$ G-coI
10	Atrophic antrum gastritis	$>$ Hp-co	$\geq$ P-coI	$<$ G-coI
	Atrophic antrum gastritis	$>$ Hp-co	$<$ P-coII	G-coI - G-coII
	Non-atrophic gastritis	$>$ Hp-co	$\geq$ P-coII	G-coI - G-coII
	Non-atrophic gastritis	$>$ Hp-co	$\geq$ P-coI	$>$ G-coII
	Atrophic corpus gastritis	$>$ Hp-co	$<$ P-coI	$>$ G-coII

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From the Table it can be seen that the *Helicobacter pylori* marker divides the set of specific combinations into two groups, wherein the measured Hp marker analytes are either above or below the cut-off value for the Hp marker (Hp-co). When the Hp-marker value is equal to or below the cut-off, a PGI value equal to or above the first PG cut-off value (P-coI) indicates a normal mucosa, whereas if the PGI value is below the said cut-off, a gastrin value equal to or above the upper cut-off limit for gastrin (G-coII) indicates atrophic corpus gastritis, whereas if this value is below the said cut-off, atrophic antrum/corpus gastritis, i.e. pangastritis is indicated. Instead of the provision of 'any value' for gastrin in the diagnosis of a normal mucosa, one can also apply a third lowest cut-off value for gastrin (G-coIII) above which the gastrin concentration should be to indicate a normal mucosa.

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On the other hand, when the Hp-value is above its cut-off value, and the gastrin value is below its lower cut-off limit (G-coI), the indication is pangastritis if also the pepsinogen I is below its first cut-off, and the indication is atrophic antrum

gastritis if the pepsinogen I is equal to or above its first cut-off. If the gastrin value is in the cut-off range (G-coI – G-coII), and the pepsinogen I is below its second cut-off value (P-coII) the indication is also atrophic antrum gastritis, but when the pepsinogen I is equal to or above its second cut-off, the indication is non-atrophic gastritis. Finally, when the gastrin is above the upper cut-off limit (G-coII), and the pepsinogen I is equal to or above the first cut-off, non-atrophic gastritis is indicated, but when the pepsinogen is below the first cut-off, atrophic corpus gastritis is indicated. In the above Table, when the presence of a Hp marker, such as the antigen, rather than the antibody concentration is determined, the table includes only the options of presence or absence of antigen in the sample.

The above mentioned diagnoses can further be supplemented with information relating to a risk of developing peptic ulcer (gastric and/or duodenal) as well as the associated risk factor therefore, as well as a possible risk of cancer, and its associated risk factor. The criteria for establishing the risk of peptic ulcer and of cancer based on the measured PGI and G-17 values as compared to their cut-off and reference values, have been described in the WO 00/ 67035 and WO 96/15456 respectively. The different risk factors associated with peptic ulcer and cancer have been described in detail for example in *Gastroenterology Clinics of North America*, Volume 29, No. 3 (2000), reference being made especially to page 586, where the various risk factors associated with normal, non-atrophied, mild, moderate and severe atrophy of the antrum and corpus mucosa are disclosed both for peptic ulcer and cancer, in diagram form.

Further optional parameters that can be taken into account, when creating the information for display for example in the form of a diagnosis, can be the age of the patient, a condition of e.g. reflux symptoms, dyspepsia, and/or anemia, or also other symptoms and disease states, whereby, according to an embodiment of the invention, the mentioned symptoms or diseases can be entered into the data processing means as boolean parameters.

The Table disclosed above can be supplemented, for example, with a cut-off value

for age, whereby, for example, an age over 45 years in combination with dyspepsia in patients with a *Helicobacter pylori* infection and a non-atrophic gastritis or atrophic corpus gastritis can, in addition to the diagnosis relating to the state of the gastric mucosa, prompt a suggestion to treat the patient for *Helico-*  
5 *bacter pylori* infection.

Furthermore, for example a diagnosis of atrophic corpus gastritis optionally in combination with pernicious anemia irrespective of age could prompt a suggestion for testing for vitamin B12 and homocysteine from the serum, and possibly also a  
10 suggestion for B12 vitamin supplementation treatment.

The invention is also directed to a kit comprising means for measuring, from a sample, the pepsinogen I and/or gastrin concentration, and/or means for  
15 determining the concentration or presence of a *Helicobacter pylori* marker. The kit thus contains the various means, such as reagents, needed for measuring the concentration or presence of the analyte in question, packed in different containers but contained in the same package. The kit also can contain instructions of use. In addition the kit contains a computer program product embodied on a computer  
20 readable medium and comprising computer code means, which when run on a computer, are adapted to perform the steps of comparing the measured concentration values of an analyte with its predetermined cut-off value to provide the combination of comparison results, which together with optionally other data entered forms the basis for the information to be generated when used on a  
25 computer. The computer program product can contain pre-stored cut-off values for one or more of the analytes to be measured, or such values can be entered at the time of use. When using the kit, the concentration of the at least one analyte, as well as the concentrations of any of the other analytes as required, measured with the same kit or using other means or kits, are entered when using the program on a  
30 computer.

Yet another object of the invention is a computer program product embodied on a computer readable medium and comprising computer code means adapted to

perform the steps of comparing the measured concentration values of an analyte, such as the analytes Hp-marker, PGI and gastrin, with its predetermined cut-off value, to provide combinations of comparison results to generate, optionally with other data entered, information, when run on a computer.

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Reference is made to the enclosed Fig. 1, wherein a diagram referring to a test panel or 'decision tree' according to an embodiment of the invention is disclosed. The cut-off values for each one of the analytes are those indicated in the diagram and referred to below, which have shown to give accurate diagnoses for the average population. Naturally it is possible to use also other or more or less modified cut-off values, should the methods used for the determination of the analyte concentrations, and/or the sensitivity and specificity so require.

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The sample used is a serum sample, and the gastrin is gastrin-17 (G-17). In the determination of serum pepsinogen I the cut-off-value for atrophic gastritis is, according to our previous studies, 20-30  $\mu\text{g/l}$  depending on the specificity and sensitivity agreed upon for the method in question, which corresponds to appr. 450 - 690 pmol/l. In this example we have used a value of 25  $\mu\text{g/l}$  as the first cut-off value for atrophic gastritis in the corpus area or atrophic pangastritis. However, according to the invention it is possible to use also a second value for PGI as a cut-off value to distinguish between atrophic gastritis in the antrum (with only mild atrophic corpus gastritis) and non-atrophic gastritis, respectively, when the G-17 is within the cut-off range for G-17. In the context of this invention, this second value is termed the second cut-off value. In this example we have used a value of 50  $\mu\text{g/l}$  for this second cut-off value, a value appr. twice that of the first cut-off value. If the pepsinogen II analyte concentration is measured in addition, the first cut-off value for the ratio of PGI to PGII can be for example 2.5, and the second cut-off for example appr. 5.

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In this example we use the serum gastrin-17 concentration obtained after protein stimulation, i.e. the postprandial gastrin-17. The cut-off range for 'stimulated' gastrin-17 (G-17st) is normally in the range of 5 to 10 pmol/l, whereby the lower

limit of 5 pmol/l forms a lower cut-off value, the upper limit of 10 pmol/l the upper cut-off value, and the intermediate range a third cut-off range. In addition there is a lowest cut-off for gastrin-17 of 0.5 pmol/l which is applicable for indicating a normal mucosa with normal PGI values ( $\text{PGI} \geq 25 \mu\text{g/l}$ ). The

5 corresponding values and ranges for unstimulated G-17 would be approximately half of those given for the stimulated values. In this example we have used the *Helicobacter pylori* antibodies as a Hp-marker, and we have found that a suitable cut-off value for the *Helicobacter pylori* antibody value is appr. 30 EIU.

10 According to the embodiment described in this example, the data processing means carries out a comparison of the measured Hp-Ab value to the cut-off value for Hp-Ab, which is indicated as being 30 EIU. Depending on the measured value being higher than, or equal to or lower than the predetermined cut-off value, the data processing means perform in the first case a comparison of the measured G-17st to

15 its cut-off range, and in the later case the data processing means perform a comparison of PGI to its first cut-off value.

If, in the first case, the G-17st is either below the lower cut-off value, or above its higher cut-off value, the data processing means perform a comparison of the PGI to its said first cut-off value. When G-17st is below its lower cut-off and the PGI is

20 below its first cut-off value, the data processing system will generate information relating to a diagnosis of atrophic antrum and corpus gastritis (pangastritis), but if the PGI is equal to or higher than the first cut-off value, the information will relate to atrophic gastritis of the antrum.

25 When G-17st is above its upper cut-off and the PGI is below the first cut-off value for PGI, the information generated will relate to atrophic gastritis of the corpus, but if the measured PGI is equal to or above the first cut-off value for PGI, the information generated will relate to non-atrophic gastritis. The same diagnosis will

30 be displayed if the measured PGI is high, and equal to or higher than the second cut-off value for PGI in combination with a G-17st in the cut-off range. If in turn, the measured PGI is below the second cut-off value for PGI, with G-17st in the

cut-off range, the diagnoses displayed will be atrophic gastritis of the antrum area.

On the other hand, if the Hp-Ab value is lower than or equal to its cut-off value, the data processing means perform a comparison of the measured PGI value with the first cut-off value for PGI. In case the measured PGI value is equal to or higher than the said cut-off value, the information generated will refer to a diagnosis of a normal mucosa at a gastrin-17st value above the third lowest cut-off which in this example is 0.5 pmol/l. However, if the said measured PGI value is below the first cut-off value, the information displayed will relate to a diagnosis of atrophic corpus gastritis when the measured G-17st is equal to or higher than the upper cut-off value, and to a diagnosis of pangastritis, when also the G-17st is low, namely below the upper cut-off limit. The atrophic corpus gastritis will in this case be of the autoimmune type.

As stated earlier, by feeding additional parameters into the data processing system, and combining them with the generated information, it is possible to provide additional information, for example in the form of suggestions for further treatments and/or further investigations, such as a suggestion to undergo endoscopy, to determine B12 vitamin and homocysteine levels, and eradication treatment of *Helicobacter pylori* infection.

The following examples are intended to illustrate the invention without restricting it in any way. The analytes measured are serum pepsinogen I, serum gastrin-17 and *Helicobacter pylori* antibodies; the cut-off values are those shown in Fig. 1. Also the age of the patient has been included as a parameter, the cut-off being 45 years. The respective diagnosis is given in each example, together with further suggestions for examinations and treatments, when applicable.

#### EXAMPLE 1

30

Serum pepsinogen I	30	μg/l
Serum gastrin-17st	20	pmol/l

Helicobacter pylori antibodies	10	EIU
Patient's age	40	years

Suggested diagnosis: NORMAL

- 5
- 1) No atrophic gastritis
  - 2) No H. pylori infection
  - 3) No risk of gastric cancer and peptic ulcer diseases (duodenal and gastric); risk factor 1X

10 Suggested further investigation and treatment:  
No further examinations (e.g. endoscopy) recommended.

#### EXAMPLE 2

15 Serum pepsinogen I	20	µg/l
Serum gastrin-17st	20	pmol/l
Helicobacter pylori antibodies	10	EIU
Patient's age	40	years

- 20 Suggested diagnosis: ATROPHIC CORPUS GASTRITIS
- 1) Peptic ulcer disease (duodenal and gastric ulcer) unlikely
  - 2) Autoimmune type atrophic corpus gastritis
  - 3) Increased risk of gastric cancer (risk factor 3-5X)

25 Suggested further investigation and treatment:  
Endoscopy advisable. Vitamin B12 and homocysteine determination recommended..  
Vitamin B12 replacement therapy, if B12 below 170 pmol/l.

#### EXAMPLE 3

30 Serum pepsinogen I	20	µg/l
Serum gastrin-17st	5	pmol/l

Helicobacter pylori antibodies 10 EIU

Patient's age 40 years

Suggested diagnosis: ATROPHIC ANTRUM/CORPUS GASTRITIS

1) Peptic ulcer disease (duodenal and gastric) unlikely

5 2) Increased risk of gastric cancer (risk factor 3-90X)

Suggested further investigation and treatment:

Endoscopy advisable. Vitamin B12 and homocysteine determination recommended.

Vitamin B12 replacement therapy, if B12 below 170 pmol/l.

10

#### EXAMPLE 4

Serum pepsinogen I 20  $\mu\text{g/l}$

Serum gastrin-17st 2 pmol/l

15 Helicobacter pylori antibodies 80 EIU

Patient's age 40 years

Suggested diagnosis: ATROPHIC ANTRUM/CORPUS GASTRITIS

1) Peptic ulcer disease (duodenal and gastric) unlikely

20 2) Increased risk of gastric cancer (risk factor 3-90X)

Suggested further investigation and treatment:

Endoscopy advisable. Vitamin B12 and homocysteine determination recommended.

Vitamin B12 replacement therapy, if B12 below 170 pmol/l. Treatment of H.

25 pylori infection necessary.

#### EXAMPLE 5

Serum pepsinogen I 30  $\mu\text{g/l}$

30 Serum gastrin-17st 2 pmol/l

Helicobacter pylori antibodies 80 EIU

Patient's age 40 years

Suggested diagnosis: ATROPHIC ANTRUM GASTRITIS

- 1) High risk of peptic ulcer disease (duodenal and gastric) (risk factor 10-20X)
- 2) Increased risk of gastric cancer (risk factor 18X)

5

Suggested further investigation and treatment:

Endoscopy advisable. Treatment of H. pylori infection necessary.

#### EXAMPLE 6

10

Serum pepsinogen I	30	µg/l
Serum gastrin-17st	8	pmol/l
Helicobacter pylori antibodies	80	EIU
Patient's age	40	years

15

Suggested diagnosis: ATROPHIC ANTRUM GASTRITIS

- 1) High risk of peptic ulcer disease (duodenal and gastric) (risk factor 10-20X)
- 2) Increased risk of gastric cancer (risk factor 18X)

20

Suggested further examination and treatment:

Endoscopy advisable. Treatment of H. pylori infection necessary.

#### EXAMPLE 7

25

Serum pepsinogen I	60	µg/l
Serum gastrin-17st	8	pmol/l
Helicobacter pylori antibodies	80	EIU
Patient's age	40	years

30

Suggested diagnosis: NON-ATROPHIC GASTRITIS

- 1) Increased risk of peptic ulcer disease (duodenal and gastric) (risk factor 10X)
- 2) Low risk of gastric cancer (risk factor 1-2X)

Suggested further investigation and treatment:  
Endoscopy not necessary. Treatment of H. pylori infection necessary.

5      **EXAMPLE 8**

	Serum pepsinogen I	50	µg/l
	Serum gastrin-17st	20	pmol/l
	Helicobacter pylori antibodies	80	EU
10	Patient's age	40	years

Suggested diagnosis: **NON-ATROPHIC GASTRITIS**  
1) Increased risk of peptic ulcer disease (duodenal and gastric) (risk factor 10X)  
2) Low risk of gastric cancer (risk factor 1-2X)

15      Suggested further investigation and treatment:  
Endoscopy not necessary. Treatment of H. pylori infection necessary.

**EXAMPLE 9**

20

	Serum pepsinogen I	20	µg/l
	Serum gastrin-17st	20	pmol/l
	Helicobacter pylori antibodies	80	EU
	Patient's age	40	years

25      Suggested diagnosis: **ATROPHIC CORPUS GASTRITIS**  
1) Increased risk of gastric cancer (risk factor 3-5X)

Suggested further investigation and treatment:  
30      Endoscopy advisable. Vitamin B12 and homocysteine determination recommended.  
Vitamin B12 replacement therapy, if B12 below 170 pmol/l. Treatment of H.  
pylori infection.

## EXAMPLE 10

5	Serum pepsinogen I	50	µg/l
	Serum gastrin-17st	20	pmol/l
	Helicobacter pylori antibodies	80	EIU
	Patient's age	50	years

10 Suggested diagnosis: NON-ATROPHIC GASTRITIS

- 1) Increased risk of peptic ulcer disease (duodenal and gastric) (risk factor 10X)
- 2) Low risk of gastric cancer (risk factor 1-2X)

Suggested further investigation and treatment:

15 Endoscopy advisable. Treatment of H. pylori infection if dyspeptic symptoms.

## EXAMPLE 11

	Serum pepsinogen I	20	µg/l
20	Serum gastrin-17st	20	pmol/l
	Helicobacter pylori antibodies	80	EIU
	Patient's age	50	years

Suggested diagnosis: ATROPHIC CORPUS GASTRITIS

- 25 1) Increased risk of gastric cancer (risk factor 3-5X)

Suggested further investigation and treatment:

Endoscopy advisable. Vitamin B12 and homocysteine determination recommended.

Vitamin B12 replacement therapy, if B12 below 170 pmol/l. Treatment of H.

30 pylori infection necessary if dyspeptic symptoms.

## CLAIMS

1. A method for assessing the condition of the gastric mucosa, especially for diagnosing mucosal gastric changes, such as atrophic gastritis, in a subject, by  
5 assaying the analytes pepsinogen I (PGI), gastrin and a marker for *Helicobacter pylori* infection, the method comprising
- measuring from a sample of said subject the pepsinogen I and gastrin concentration, and, in addition, determining the concentration or presence of a marker for *Helicobacter pylori* (Hp-marker), and
  - 10 - entering the data so obtained for said analytes in a data processing means comprising an operating system, means for transceiving and processing data, said data processing means being adapted to perform the steps of
  - comparing a measured concentration value for an analyte to a predetermined cut-off value for said analyte, to obtain a combination of comparison results which is  
15 specific for the subject tested, and generating information in response to the said combination of comparison results, and optionally other data entered.
2. The method according to claim 1, wherein the *Helicobacter pylori* marker is a *Helicobacter pylori* antibody, the concentration of which is measured from the  
20 sample.
3. The method according to claim 1, wherein the *Helicobacter pylori* marker is the *Helicobacter pylori* antigen, the presence of which is determined in the sample.
- 25 4. The method according to any one of the preceding claims, wherein the gastrin value measured is the gastrin-17 (G-17), especially the stimulated gastrin-17 value (G-17st), or both the gastrin-17 and the stimulated gastrin-17.
5. The method according to any one of the preceding claims, wherein, in addition,  
30 the concentration of the analyte pepsinogen II (PGII) is measured, and the ratio PGI/PGII is formed for comparison.

6. The method according to claim 2, wherein the analytes are measured from a body fluid, such as a serum, urine, saliva or lacrimal fluid sample, especially a serum sample.
- 5      7. The method according to any one of the preceding claims, wherein the data processing means comprise a display, and the information generated is displayed on the display.
- 10      8. The method according to any one of the preceding claims, wherein the information generated comprises diagnostic information relating to mucosal gastric changes.
- 15      9. The method according to any one of the preceding claims, wherein the information generated comprises suggestions for further treatment or for further investigations.
- 20      10. The method according to any one of the preceding claims 8 - 9, wherein the information indicates the risk of peptic ulcer and/or cancer disease, and optionally of the associated risk factor.
- 25      11. The method according to any one of the preceding claims, wherein additional data relating to the age of the patient, reflux symptoms, dyspepsia and/or anemia in the subject is entered as parameters.
- 30      12. The method according to any of the preceding claims, wherein the predetermined cut-off values for PGI and gastrin and optionally the Hp-marker and PGII are entered into the data processing means using means for data input and storing the data in storing means.
- 30      13. A kit comprising means for determining, from a sample, the pepsinogen I and/or gastrin concentration, and/or the concentration or presence of a *Helicobacter pylori* marker, as well as a computer program product embodied on a

computer readable medium and comprising computer code means adapted to perform the steps of comparing a determined concentration value of an analyte to a predetermined cut-off value for said analyte, combining the results of comparison to a combination of comparison results, and providing information in response to said combination and optionally to other entered data, when run on a computer.

14. The kit according to claim 13, wherein the analytes to be determined and entered into the data processing system for comparison by the computer code means comprise a Hp-marker, PGI and gastrin, and wherein predetermined cut-off values for said analytes are stored on the computer readable medium containing the computer program and for use with the computer program, for use in a method according to any one of the preceding claims 1 to 12.

15. A computer program product embodied on a computer readable medium and comprising computer code means adapted to perform the steps of comparing measured concentrations of an analyte to a respective cut-off value for said analyte, combining the results of comparison to a combination of comparison results, and providing information in response to said combination and optionally to other entered data, when run on a computer.

16. The computer program product according to claim 15 wherein the analytes comprise a Hp-marker, PGI and gastrin, the product also comprising data for predetermined cut-off values for said analytes, for use in the method of any one of the preceding claims 1 to 12.

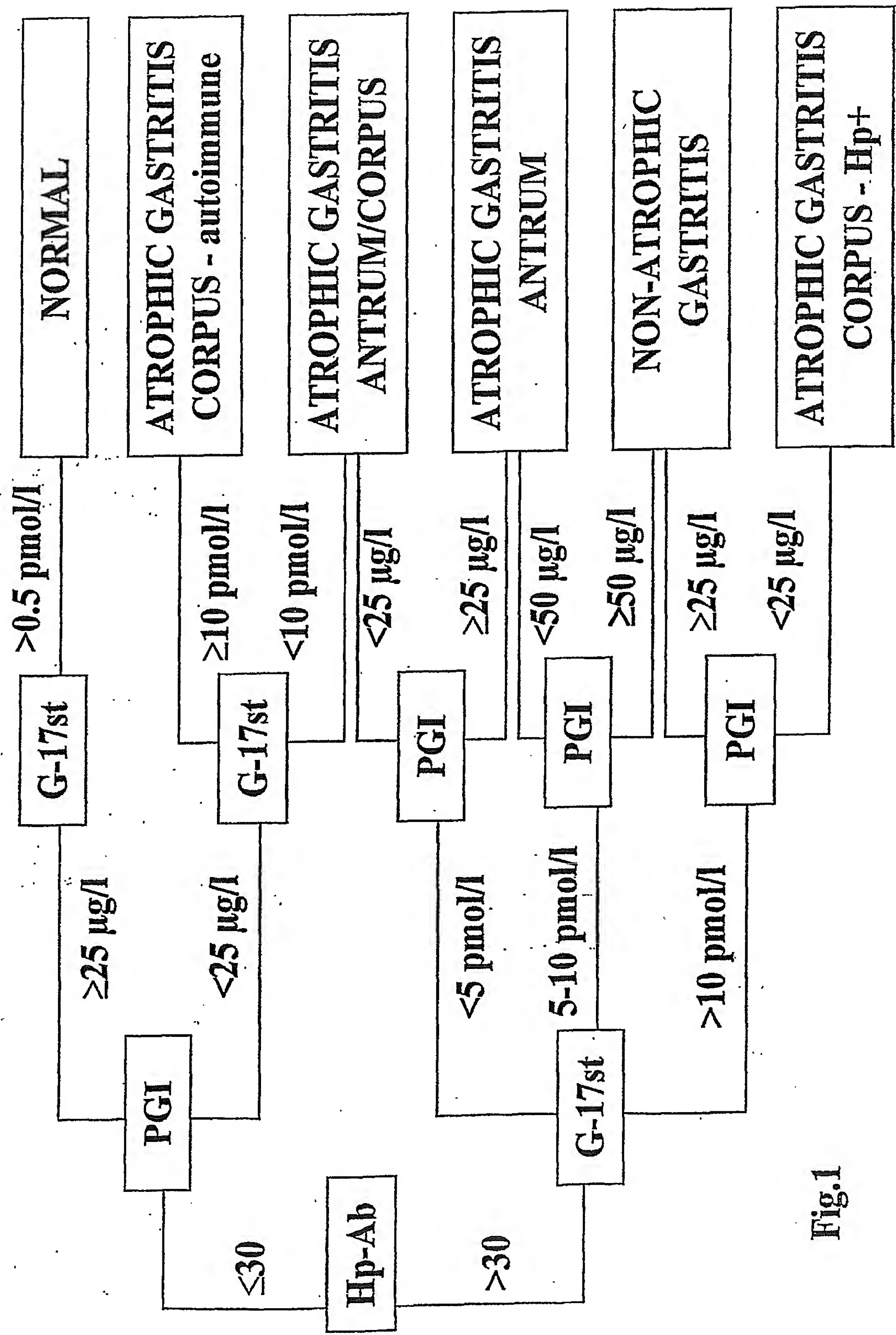


Fig.1

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 02/00008

## A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/74, G01N 33/573

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI DATA, PAJ, MEDLINE, BIOSIS, INSPEC

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9615456 A1 (LOCUS GENEX OY), 23 May 1996 (23.05.96), the whole document	1-4,6-16
Y	--	5
X	WO 0067035 A1 (LOCUS GENEX OY), 9 November 2000 (09.11.00), the whole document, especially page 5, line 5-8 and 26-31	1-4,6-16
Y	--	5

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

11 April 2002

Date of mailing of the international search report

17-04-2002

Name and mailing address of the ISA/  
Swedish Patent Office  
Box 5055, S-102 42 STOCKHOLM  
Facsimile No. +46 8 666 02 86

Authorized officer

Jens Waltin/MP  
Telephone No. +46 8 782 25 00

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 02/00008

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Cancer Epidemiology, Biomarkers & Prevention, Volume 10, February 2001, Catherine Ley et al, "Screening Markers Chronic Atrophic Gastritis in Chiapas, Mexico", page 107 - page 112, see especially Abstract and Introduction, page 107 and table 1 & 2	5
A	--	1-4,6-16
X	Journal of Gastroenterology, Volume 30, 1995, Jaw-Town Lin et al, "Diagnosis of gastric adenocarcinoma using a scoring system: Combined assay of serological markers of Helicobacter pylori infection, pepsinogen I and gastrin", page 156 - page 161, see especially Introduction and page 160	1-16
X	--	
X	European Journal of Gastroenterology & Hepatology, Volume 8, 1996, Ernst J. Kuipers et al, "Helicobacter pylori, pepsinogens and gastrin: relationship with age and development of atrophic gastritis", page 153 - page 156, see especially table 1 and page 155, col 2, last paragraph	1-16
X	--	
X	The American Journal of Gastroenterology, Volume 89, No 8, 1994, Siegfried Wagner et al, "Helicobacter pylori Infection and Serum Pepsinogen A, Pepsinogen C, and Gastrin in Gastritis and Peptic Ulcer: Significance of Inflammation and Effect of Bacterial Eradication", see discussion and table 1	1-16
A	--	
A	EP 0760482 A2 (FURUTA, TAKAHISA, DR.), 5 March 1997 (05.03.97), page 3, line 1 - line 30	5
	-- -----	

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/FI02/00008

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
**Claims 1-12 seem to relate to a diagnostic method practised on the human body (Rule 39.1.(iv)). Nevertheless, a search has been executed for these claims.**
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest** ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

28/01/02

International application No.

PCT/FI 02/00008

Patent document cited in search report			Publication date	Patent family member(s)		Publication date
WO	9615456	A1	23/05/96	AT	213332 T	15/02/02
				AU	3874395 A	06/06/96
				DE	69525476 D	00/00/00
				EP	0804737 A,B	05/11/97
				FI	97304 B,C	15/08/96
				FI	945391 A	17/05/96
				JP	10509795 T	22/09/98
				US	2001039025 A	08/11/01
WO	0067035	A1	09/11/00	AU	4299800 A	17/11/00
				EP	1173770 A	23/01/02
				FI	990992 A	31/10/00
EP	0760482	A2	05/03/97	JP	2949474 B	13/09/99
				JP	9061428 A	07/03/97
				US	6156525 A	05/12/00